치의학박사학위논문

Real-time measurement of dentinal fluid flow during laser irradiation and desensitizing agents application

레이저 조사 및 탈감작제 적용에 따른 상아세관액 흐름의 실시간 측정

2015년 8월

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치의과학과 치과보존학 전공

김 민 호

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이 논문을 치의학박사학위 논문으로 제출함

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김 민 호

김민호의 박사학위 논문을 인준함

2015년 7월

위 원 장 ________________ (인)

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위 원 ________________ (인)

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Abstract

Real-time measurement of dentinal fluid flow during laser irradiation and desensitizing agents application

Min-Ho Kim

Program in Conservative Dentistry

Department of Dental Science

Graduate School, Seoul National University

(Directed by Professor In-Bog Lee, D.D.S., M.S.D., Ph.D.)

Objectives. The objective of this study was to evaluate the effects of lasers (Nd:YAG and Er:YAG) and of topical desensitizing agents on dentinal tubule occlusion by measuring real-time dentinal fluid flow (DFF).

Methods. Thirty-two molars were prepared with V-shape cavity at the cervical area, acid-etched, water rinsed, blotted dry, and treated with 1) Nd:YAG laser; 2) Er:YAG laser; 3) SuperSeal, a desensitizing agent; 4) ClinproXT, a resin modified glass ionomer (RMGI) varnish (n=8 each). A real-time fluid flow measuring instrument (nano-Flow) was used to measure the DFF throughout the procedures.

The DFF rates before and after the treatment were compared. Moreover, the surface topography of dentinal tubules after each desensitizing method was examined using SEM.

Results. DFF varied among the groups. The DFF rate was significantly reduced after laser irradiation/application of the desensitizing agents ($p<0.05$). ClinproXT showed the greatest reduction of DFF rate (71.9%), followed by SuperSeal (34.8%) and laser groups ($p<0.05$). However, there was no significant difference between the Nd:YAG (24.1%) and Er:YAG (20.6%) groups ($p>0.05$). In SEM images, narrowed dentinal tubules were observed in both lased groups and SuperSeal group. In ClinproXT group, the occluded dentinal tubules by the RMGI covering were observed.

Conclusion. The fluid flow measuring instrument could clearly show the changes of DFF rate during laser irradiation/application of the desensitizing agents in real-time. ClinproXT, a RMGI varnish and SuperSeal, an oxalate-based desensitizing agent showed better results than the Nd:YAG and Er:YAG lasers in the reduction of DFF rate.

Keywords: Dentinal fluid flow, Real-time measurement, Nd:YAG laser, Er:YAG laser, Desensitizing agent

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Real-time measurement of dentinal fluid flow during laser irradiation and desensitizing agents application

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Introduction

Dentin hypersensitivity is characterized by an acute, and short-term pain, which is induced by mechanical, thermal, chemical, or osmotic stimulus after exposure of dentinal tubules to oral environment[1]. The most widely accepted explanation for dentin hypersensitivity is Brännström's "hydrodynamic theory", which states that stimulus-induced movement of fluid within exposed dentinal tubules stimulates pulpal mechanoreceptors[2]. Thus, pain can be prevented by total or partial occlusion of the exposed dentinal tubules[3].

Based on this theory, several methods for occluding dentinal tubules have been introduced, of which the application of desensitizing agents is widely recommended. The most commonly used desensitizing agents for dentin hypersensitivity are intended to seal the dentin surface by protein precipitation[4], calcium complex formation[5], or oxalate obstruction[6]. Dentin bonding agents[7] and resin-modified glass ionomer (RMGI)[8] are also used for the treatment of dentin hypersensitivity. Recently, lasers were described to effectively achieve dentinal tubule occlusion[9]. Several explanations for the effects of laser irradiation in the treatment of dentin hypersensitivity have been proposed, including an analgesic effect related to depressed nerve transmission, obliteration of the dentinal tubules via tertiary dentin production, and sealing of dentinal tubules by dentin melting and recrystallization; the last explanation is most widely accepted[10].

The neodymium-doped: yttrium, aluminium and garnet (Nd:YAG) laser is a near-infrared laser that emits light at a wavelength of 1064 nm. This laser has been used in dentistry since the 1970s and has been reported to penetrate deep into the tooth structure and melt the hydroxyapatite crystals, thereby occluding the dentinal tubules[11]. In contrast, the erbium-doped: yttrium, aluminium and garnet (Er:YAG) laser emits light at a wavelength of 2940 nm and has been shown to generate less heat and be more absorbed by water than the Nd:YAG laser[12]. Some clinical studies have reported good outcomes in the treatment of dentin hypersensitivity with Er:YAG lasers[13, 14].

Soluble potassium oxalate makes oxalate ions that can react with calcium ions in dentin to form insoluble calcium oxalate crystals that are deposited in the dentinal tubules[15]. In−vitro studies have shown the effectiveness of oxalate in occluding the dentinal tubules[16, 17].

Glass ionomer has been long used for the treatment of dentin hypersensitivity[18], recently, a new paste−liquid type of RMGI has been introduced and reported to show clinically favorable outcome[19].

However, the studies that have evaluated the effectiveness of laser or desensitizing agent on dentin hypersensitivity have been mainly either clinical studies comparing the degree of symptom relief[20−22], or in vitro studies examining dentinal tubule occlusion by SEM[16, 23]. Since dentinal fluid flow (DFF) induced by external stimuli is a crucial factor associated with dentin hypersensitivity, an evaluation of changes in DFF would provide valuable information for predicting the effectiveness of various desensitizing procedures (i.e. lasers or topical agents) on dentinal tubule occlusion and pain relief.

A recent study measured real−time DFF during amalgam and composite restoration on extracted teeth[24]. In this study, a fluid flow though the dentinal tubules was traced with subnanoliter resolution using a photosensor and servomotor. Although some studies have measured changes in dentin permeability after the application of desensitizing agents[25, 26], no study has yet investigated dentin permeability and DFF changes in real−time during laser irradiation. Therefore, a comprehensive evaluation of DFF changes would provide important information in establishing the effectiveness of laser irradiation and desensitizing agents application in the treatment of dentin hypersensitivity.

The objective of this study was to measure the changes of DFF in real−time and to compare the amounts of dentinal tubule occlusion achieved with a Nd:YAG laser, an Er:YAG laser, an oxalate−based desensitizing agent, and a RMGI varnish. The null hypothesis of this study was that laser irradiation or application of a desensitizing agent on the exposed dentin does not result in any changes in DFF.

Materials and Methods

Structure and working principle of the real−time fluid flow measuring instrument

The real−time fluid flow measuring instrument (nano−Flow, IB Systems, Seoul, Korea) was used as in previous studies[17, 24]. This instrument consists of three parts: a glass capillary and photosensor for detecting fluid movement; a servomotor, lead screw and ball nut for tracking fluid movement; and a rotary encoder and computer software for data recording (Fig. 1).

An air bubble was created in a water−filled glass capillary with an internal diameter of 0.5 mm, which connected the tooth to a water reservoir. A photosensor consisting of an infrared light−emitting diode and a photo transistor detected the movement of the air bubble in the capillary as the water flowed. The servo amplifier and servomotor rotated a lead screw according to the output voltage from the photosensor to track the water−air interface continuously. The rotation of the screw was measured using a rotatory encoder, which detected 1,000 pulses per rotation, and the number of pulses was stored on a computer. The minimum measurable volume of water movement in this instrument was 0.2 nL.

Specimen preparation

Thirty-two caries or restoration-free molars were used in this study. Extracted teeth were stored in a 1% chloramine-T solution at 4 ℃ and were used within three months after their extraction. Each root was removed at 5 mm below the cemento-enamel junction. The pulp tissue in the pulp chamber was carefully removed using thin tissue forceps and endodontic files. A sandblasted slide glass with a hole drilled in its center was used to mount each tooth. A metal tube with a diameter of 0.9 mm was inserted into the hole to connect the tooth through a silicone tubing and a glass capillary to the water reservoir, and the slide glass was attached to the tooth using a dentin bonding agent (Adper Scotchbond Multi Purpose, 3M ESPE, St. Paul, MN, USA) and a flowable composite (Denflow, Vericom, Anyang, Korea). The exposed root surface and outer surface of the bonded interface between the slide glass and the tooth were covered with nail varnish to prevent undesirable leakage. The prepared specimen was connected to the measuring instrument; finally, the absence of leakage under a hydrostatic pressure of 20 cm H_2O was confirmed.

Measurement of dentinal fluid flow during laser irradiation/ desensitizing agent application

Each specimen was connected to the measuring instrument and allowed to stabilize for 10 min at a hydrostatic pressure of 20 cm H_2O to simulate physiological pulpal pressure. A V–shaped cavity with a mesio–distal width of 5 mm, an occluso–cervical height of 3 mm, and a depth of 2 mm was prepared with a high–speed handpiece (MACH–QD, NSK, Tokyo, Japan) and a round–end tapered diamond bur (TR–12, Mani, Tochigi, Japan) at the cervical cemento–enamel junction. The cavity was etched with 32% phosphoric acid (Scotchbond Universal Etchant, 3M ESPE) for 15 s to remove the smear layer, rinsed with water, and then blot–dried with a cotton pellet. The DFF was continuously measured during the whole procedures in real–time (Fig. 2).

All specimens were randomly divided into four groups of eight according to the desensitizing method (Table) as follows:

Group 1: An Nd:YAG laser was used to irradiate the specimen at a wavelength of 1064 nm, a pulse rate of 10 Hz, and an energy level of 100 mJ for 2 min with continuous flowing motion using a 300 μm fiber optic tip placed 2–3 mm away from the tooth as the manufacturer's recommendations.

Group 2: An Er:YAG laser was used to irradiate the specimen at a wavelength of 2940 nm, a pulse rate of 20 Hz, and an energy level of 20 mJ for 2 min with continuous flowing motion using a 1.5 mm fiber optic tip placed 2–3 mm away from the tooth, as per the manufacturer's recommendations.

Group 3: SuperSeal, an oxalate–based topical desensitizing agent, was applied for 30 s and gently air dried for 30 s according to the manufacturer's instructions.

Group 4: ClinproXT, a RMGI varnish, was applied as the manufacturer's instructions and light cured with a light curing unit (Elipar S10, 3M ESPE, St Paul, MN, USA).

For the laser groups, an Anybeam TOP EN laser (B&B Systems, Seoul, Korea) was used. This laser can be operated in either an Nd:YAG or an Er:YAG mode.

The DFF was continuously measured until 10 min after the laser irradiation/application of desensitizing agent. From the DFF curve, pre (as a baseline flow rate) and post application DFF rate were determined. The reduction of DFF rate was calculated as follows: Reduction of DFF rate (%) = 100 × (baseline flow rate − post application flow rate)/baseline flow rate (n=8 each).

SEM evaluation

The occlusal surfaces of the extracted molars were ground with a high-speed handpiece and a round-end tapered diamond bur to expose the dentinal tubules, after which simulated pulpal pressure was applied by connecting the teeth to the water reservoir positioned 20 cm above the teeth for 10 min. To remove the smear layer, teeth were etched with 32% phosphoric acid for 15 s and rinsed with water.

After confirming the fluid flow through the exposed dentin, each tooth was treated with one of the four desensitizing methods. The teeth were then sectioned horizontally, 3 mm below the occlusal treated surface, using a high speed handpiece. The dentin disk from each group was fractured perpendicularly. The specimens were dried in a critical point dryer (HCP−2, Hitachi, Tokyo, Japan), gold−sputtered and observed with an SEM (S−4700, Hitachi, Tokyo, Japan) at an accelerating voltage of 15kV and ×3,000 magnification.

Statistic analysis

A paired *t*−test was conducted to analyze the significance of differences in the DFF rate before vs. after laser irradiation/application of desensitizing agent. To analyze whether the DFF rate reductions were significantly different between the four groups, one−way analysis of variance was performed followed by Tukey's HSD post−hoc test ($\alpha < 0.05$).

Results

Representative curves of DFF as a function of time for the four groups were shown in Fig. 2. All groups exhibited negative flow (inward movement) during cavity preparation and washing, followed by positive flow (outward movement) during etching and blot drying. The changes of DFF in each group varied according to the application method. In the Nd:YAG group, steep negative flow was observed during the laser application, followed by steep positive flow after the application and then consistent flow with a slightly decreased slope. In the Er:YAG group, the slope of flow increased slightly during the laser application and then decreased after application when compared with that before laser irradiation. During SuperSeal application, fluid movement fluctuated and then gradually decreased in slope. In the ClinproXT group, a steep negative curve was showed during the light curing, followed by a positive flow with a decreased slope after finishing light curing.

All groups exhibited a significant reduction in the DFF rate ($p<0.05$). The DFF rate reductions after laser irradiation/application of a desensitizing agent were shown in Fig. 3. ClinproXT showed the greatest reduction of DFF rate (71.9%), followed by SuperSeal (34.8%) and laser groups ($p<0.05$). However, there was no significant difference between the Nd:YAG (24.1%) and Er:YAG (20.6%) groups ($p>0.05$).

Varying degrees of surface changes among the groups were observed in the SEM images (Fig. 4). The dentinal tubules became narrower after laser irradiation in the Nd:YAG and Er:YAG groups. Moreover, longitudinal SEM images revealed that the superficial surface of the dentinal tubules was melted by the laser energy. In the SuperSeal group, the tubules were narrowed or occluded with the many tiny crystals (0.5−2 μm) that occupied the dentinal tubules. In the ClinproXT group, RMGI covering occluded the dentinal tubules and the resin tag penetrated into the tubules.

Discussion

This study is the first to measure a continuous change in DFF during laser irradiation on exposed dentin. Various treatment modalities for dentin hypersensitivity aim to occlude the dentinal tubules in order to obstruct the movement of dentinal fluid. However, the visual evaluation of dentinal tubules by SEM is limited in that reductions in DFF cannot be quantitated. In addition, the evaluation of dentin permeability using a dentin disc, a commonly employed method, does not allow real-time measurement of dentinal fluid movement. In the present study, pulpal pressure was simulated in extracted teeth by applying a hydrostatic pressure of 20 cm H_2O and a cervical cavity preparation was made to reproduce the clinical situation. Furthermore, DFF was measured with a real-time fluid flow measuring instrument throughout all procedures, including cavity preparation, acid etching, washing, drying and laser treatment/desensitizing agent application. Thus, the effect of the desensitizing methods on DFF reduction could be evaluated in real time.

The DFF of each specimen after connecting to the measuring instrument was examined to exclude any specimens with leakage before cavity preparation. At the beginning of cervical cavity preparation, an inward DFF was evident due to the water pressure from the high-speed handpiece, since the dentinal tubules had become exposed. After cavity preparation, an outward DFF was observed through the exposed dentinal tubules when the etchant was applied, after which an inward flow was observed again due to the water pressure resulting from the rinsing process. After removal of the smear layer by the etchant, an outward flow occurred through the open dentinal tubules. Upon reaching a constant steady outward flow after completion of the pretreatment, the baseline flow rate was determined prior to laser irradiation/desensitizing agent application.

When applied to dentin, the thermal energy of the Nd:YAG laser causes occlusion or narrowing of the dentinal tubules[27]. In this study, there was a transient period of steep negative flow during lasing due to the reverse current in the capillary generated by the thermal expansion of water in the pulp chamber. When the lasing ceased, a compensating positive flow was observed as the expanded water recovered. The DFF then became stable and the flow rate was reduced by 24.1% compared with the baseline flow rate, as the permeability of the dentinal tubules was reduced by irradiation with the Nd:YAG laser.

Upon exposure to the Er:YAG laser, the lased water in the target tissue evaporates. This phenomenon is attributed to the high water absorption property of the Er:YAG laser, creating high steam pressure that in turn causes a micro-explosion of the tooth tissue[28]. Since the extent of explosion is dependent on the water content, a crater-like surface of the exposed dentinal tubule orifices often appears when the laser is applied onto the dentin[29]. The dentinal tubule size has been proposed to decrease as a result of the fusion of hydroxyapatite fragments, which are formed after laser application[30]. The actual DFF rate was reduced by 20.6 % in the present study. Unlike the Nd:YAG laser, no dramatic change in DFF due to the thermal effect from the laser application were observed, which could be explained by that the Er:YAG laser was irradiated with spraying water coolant.

The representative SEM images revealed that each laser modified the dentin surface by melting the superficial dentinal tubules, which were narrowed or occluded by irradiation with the laser. Since the melted dentin had a thickness of 3−4 μm, the laser energy does not appear to penetrate deep into the dentin[23].

SuperSeal is a potassium oxalate−based desensitizer that reacts with calcium ions of the dentin to form calcium oxalate crystals, thus resulting in occlusion of exposed dentinal tubules[17]. In the present study, the DFF rate was reduced by 34.8% after the application of SuperSeal and the occlusion of dentinal tubules by oxalate crystals was visible on the SEM images.

RMGI varnish could block the dentinal tubules by micromechanical bonding between hybrid layer and resin tag[19], and chemical bonding via ionic carboxylate bond[31]. It has been reported that 70% of RMGI may remain within the tubules, and the symptoms may be improved as a result of the presence of an amorphous zone even when 80−90% of RMGI is lost[32]. In ClinproXT varnish group, heat from the light−curing unit caused thermal expansion of water within the pulp chamber, which resulted in a transient negative fluid flow during light curing. After finishing light curing, positive fluid flow was observed again as the expanded water recovered and the fluid flow was then stabilized. The present study demonstrated 71.9% reduction in the DFF rate following ClinproXT application, and the result implies that the surface coverage with RMGI could seal effectively the dentinal tubules. SEM images confirmed a thick layer of RMGI covering the dentinal tubule. It was observed in the fractured specimen that RMGI filled the dentinal tubules, which in turn were either narrowed or occluded.

The significant reductions in DFF rate observed with all four modes of desensitizer application on the exposed open dentinal tubules signify that these four methods may be suitable for treating dentin hypersensitivity. However, none of the desensitizing methods resulted in complete occlusion of dentinal tubules, although partial occlusion with reduction of DFF was observed.

Inter-group comparisons did not reveal any significant differences between the Nd:YAG and Er:YAG lasers regarding DFF rate reduction. Although the two lasers have different mechanisms of operation, they do not appear to have different effects on DFF reduction and may even be clinically effective. But SuperSeal, a potassium oxalate-based desensitizing agent, and ClinproXT, a RMGI varnish, significantly reduced the DFF rate compared with the laser groups.

Laser irradiation or desensitizer application on exposed dentin reduced the DFF by narrowing the dentinal tubules. However, it is not yet known how long this reduction in DFF persists. From a clinical perspective, the dentin treated with desensitizing methods will always be in contact with moisture and will also have abrasions due to brushing. Further studies are required to evaluate the changes of DFF over time and upon brushing of lased or desensitizer applied dentin.

Conclusion

The fluid flow measuring instrument could clearly show the changes of DFF rate during laser irradiation/application of the desensitizing agents in real-time. ClinproXT, a RMGI varnish and SuperSeal, an oxalate-based desensitizing agent showed better results than did the Nd:YAG and Er:YAG lasers in terms of the reduction of DFF rate.

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Table and Figures

Table. Desensitizing methods used in this study

Desensitizing method	Specification or Component	Application method	Manufacturer
Nd:YAG laser	Wavelength 1064 nm Frequency 10 Hz Energy 100 mJ Output power 1.0 W	2 min. using 300 μm diameter tip	B&B systems, Seoul, Korea
Er:YAG laser	Wavelength 2940 nm Frequency 20 Hz Energy 20 mJ Output power 0.4 W	2 min. using 1.5 mm diameter tip	B&B systems, Seoul, Korea
SuperSeal	Oxalate, potassium salt	Apply 30 s (rubbing), gently air dry 30 s	Phoenix Dental, Fenton, MI, USA
ClinproXT	Liquid: HEMA, water, initiators and calcium gly-cerophosphate Paste: HEMA, Bis-GMA, water, initiators and fluo-roaluminosilicate glass	Mix 15 s, apply a thin layer (0.5 mm), light curing 20 s	3M ESPE, St Paul, MN, USA

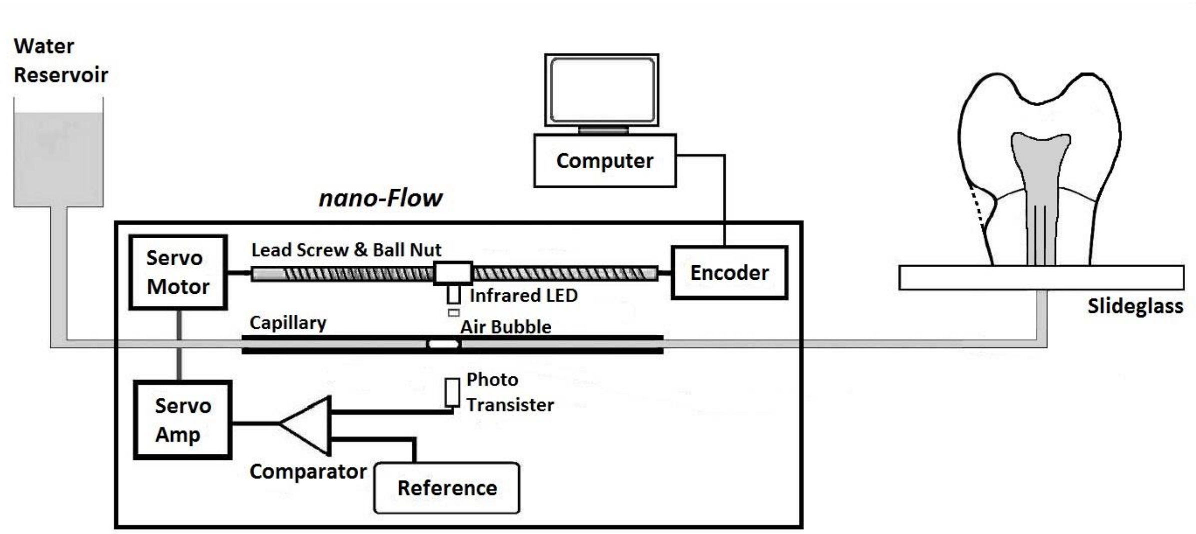
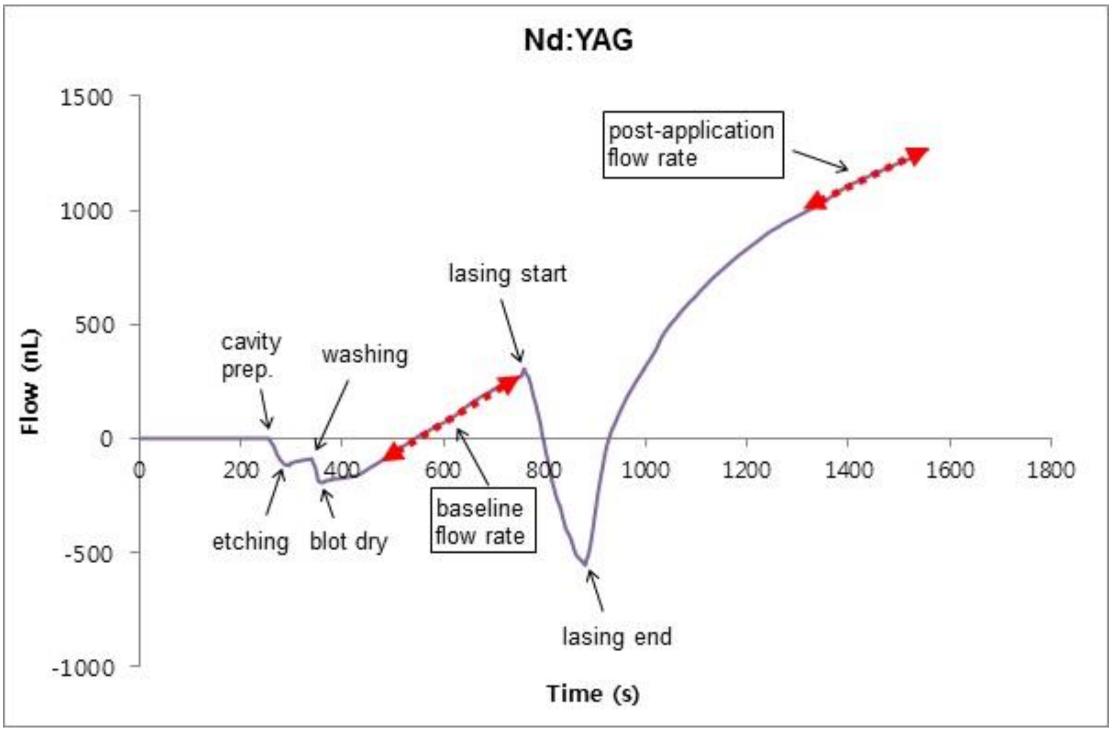
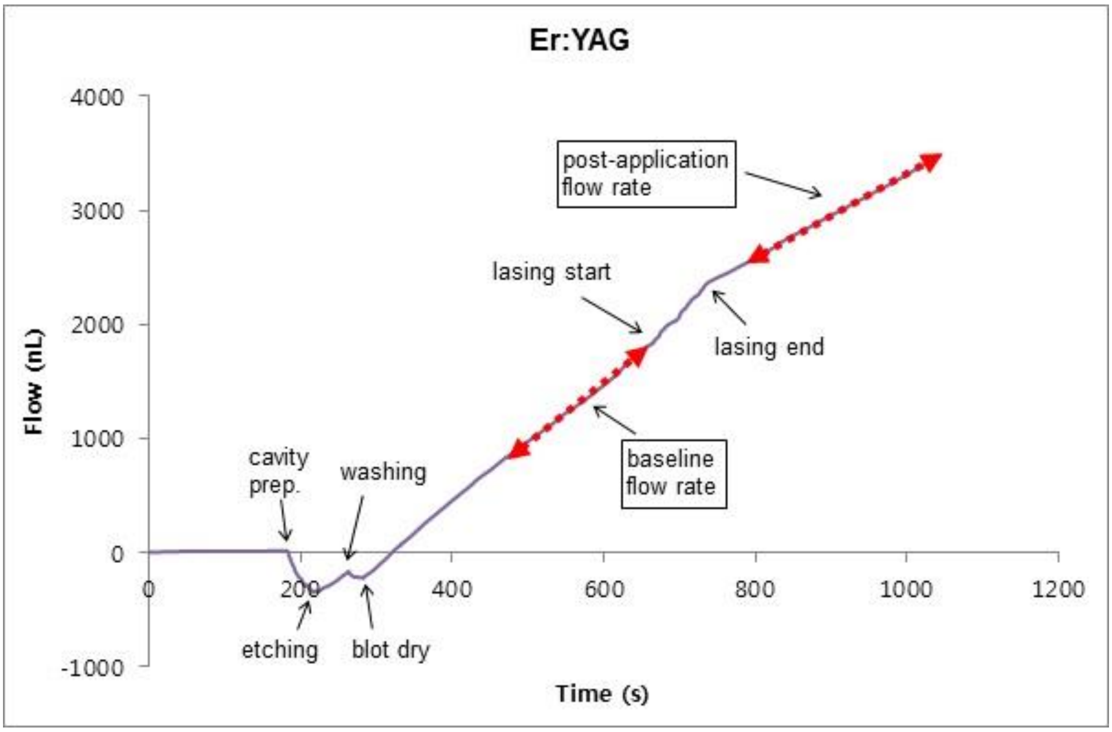


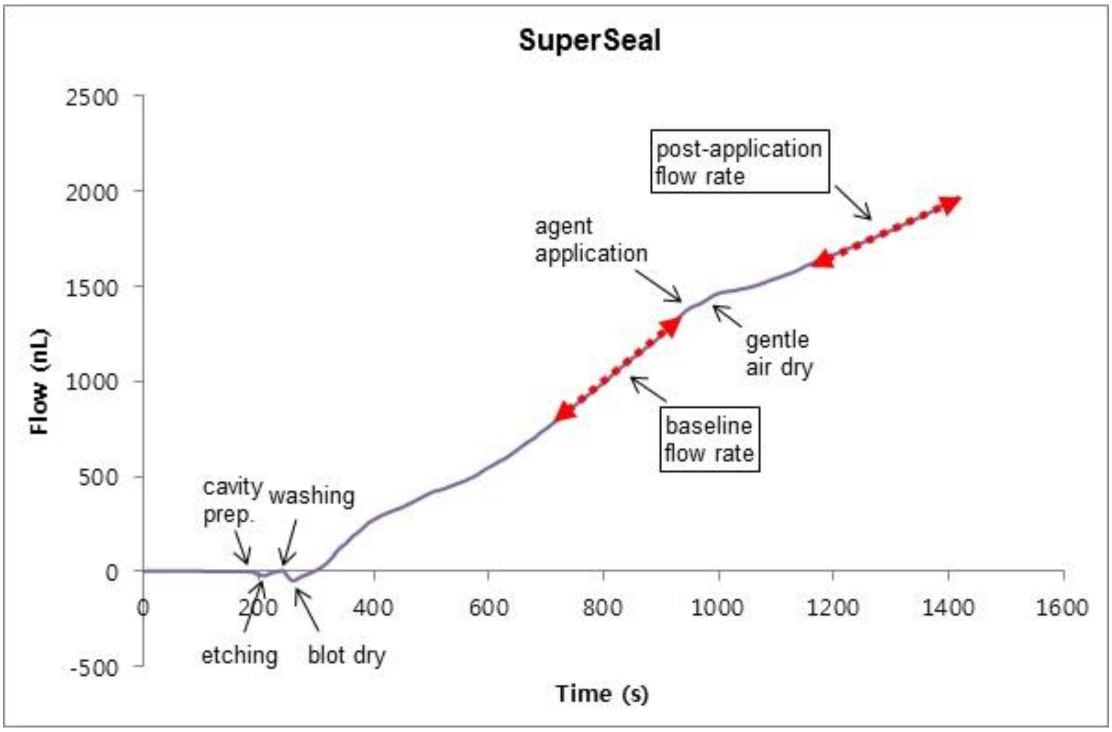
Figure 1. Schematic diagram of the subnanoliter-scaled dentinal fluid flow measurement instrument (nano-Flow).


Nd:YAG
1500
1000
500
0
-500
-1000
Flow (nL)
0
200
400
600
800
1000
1200
1400
1600
1800
Time (s)
cavity
prep.
washing
etching
blot dry
baseline
flow rate
lasing start
lasing end
post-application
flow rate

(a)

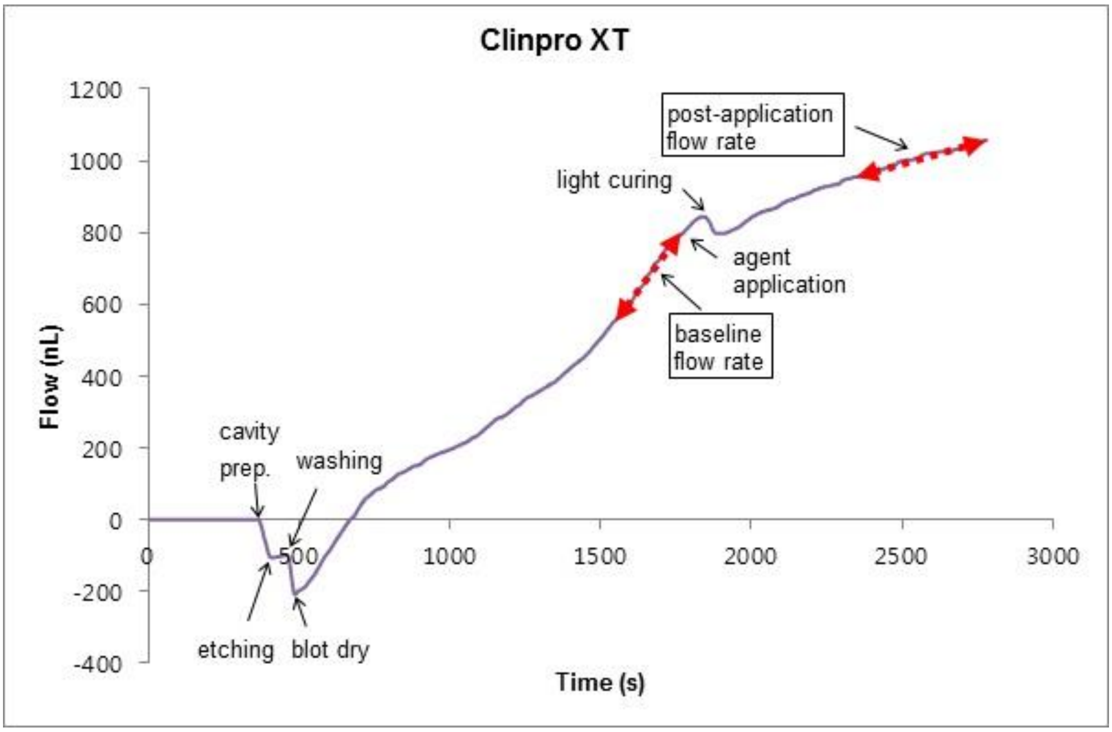

Er:YAG
4000
3000
2000
1000
0
-1000
Flow (nL)
0
200
400
600
800
1000
1200
Time (s)
cavity
prep.
washing
etching
blot dry
baseline
flow rate
lasing start
lasing end
post-application
flow rate

(b)



SuperSeal
Flow (nL)
Time (s)
2500
2000
1500
1000
500
0
-500
0
200
400
600
800
1000
1200
1400
1600
post-application
flow rate
agent
application
gentle
air dry
baseline
flow rate
cavity
prep.
washing
etching
blot dry

(c)



Clinpro XT
Flow (nL)
Time (s)
1200
1000
800
600
400
200
0
-200
-400
0
500
1000
1500
2000
2500
3000
post-application
flow rate
light curing
agent
application
baseline
flow rate
cavity
prep.
washing
etching
blot dry

(d)

Figure 2. Dentinal fluid flow as a function of time throughout the procedures with laser irradiation or the application of a desensitizing agent. (a) Nd:YAG laser (10 Hz, 100 mJ, 2 min). (b) Er:YAG laser (20 Hz, 20 mJ, 2 min). (c) SuperSeal. (d) ClinproXT.

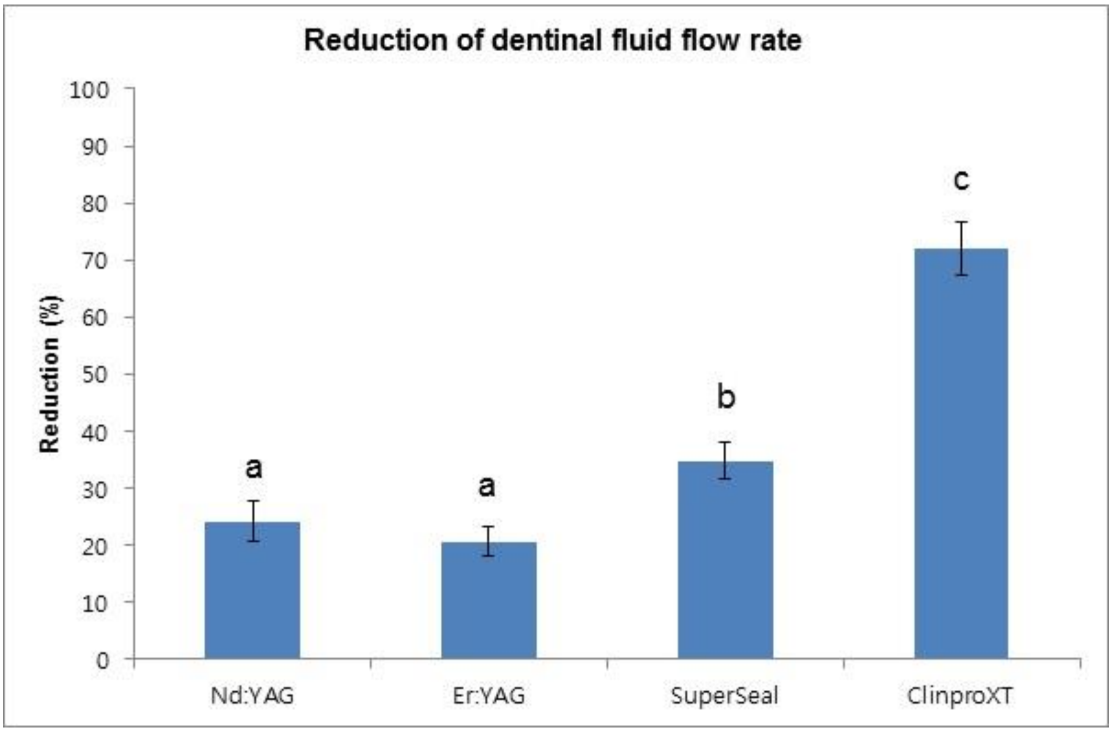
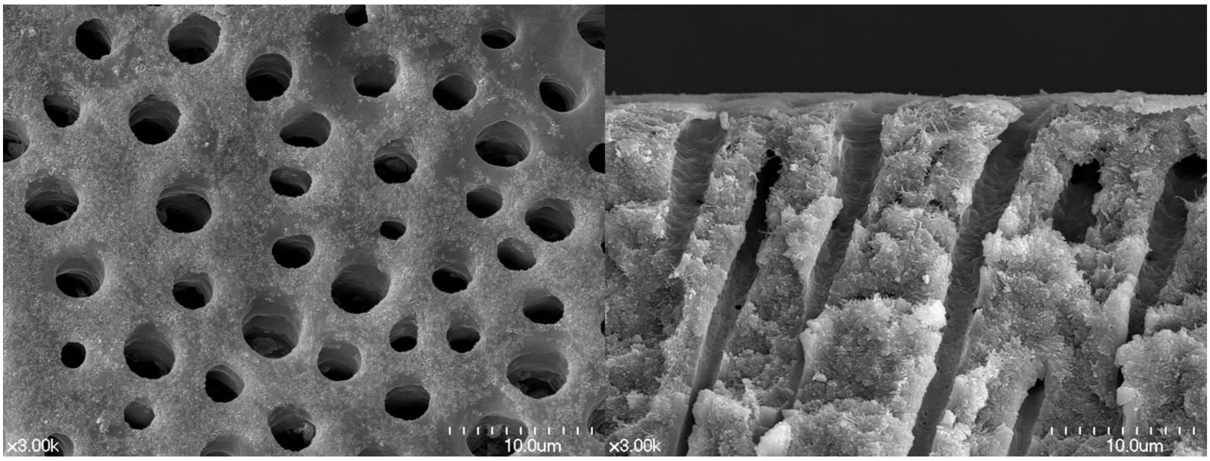
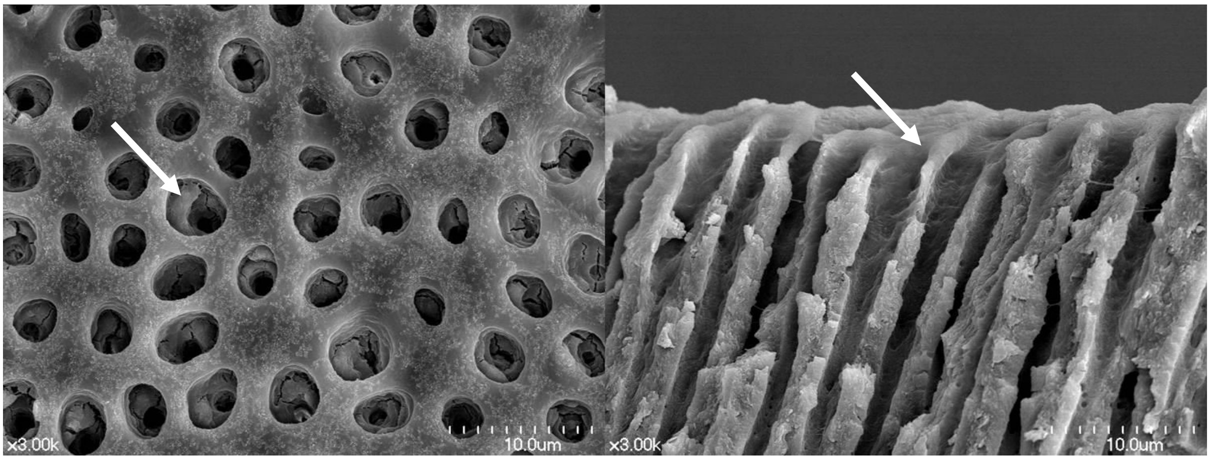


Figure 3. Reductions (%) of dentinal fluid flow rate by the two lasers and the desensitizing agents.

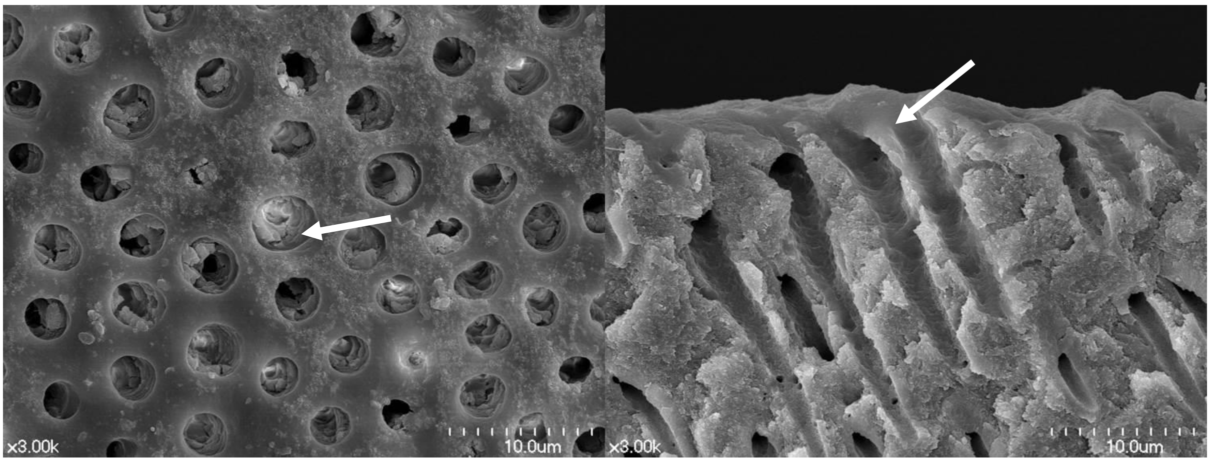
Groups designated with the same letter were not significantly different ($p>0.05$).


x3.00k
10.0um
x3.00k
10.0um

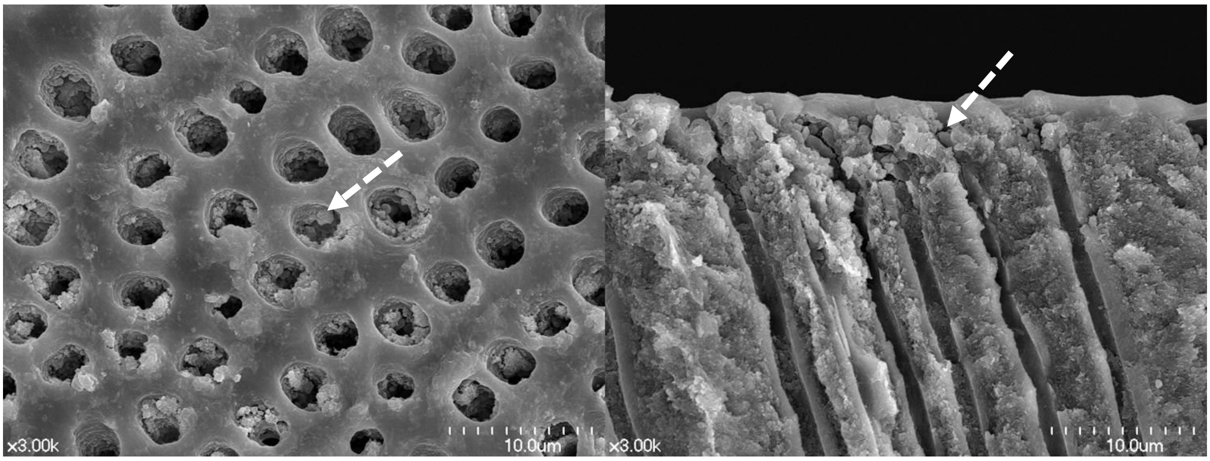
(a)


x3.00k
10.0um
x3.00k
10.0um

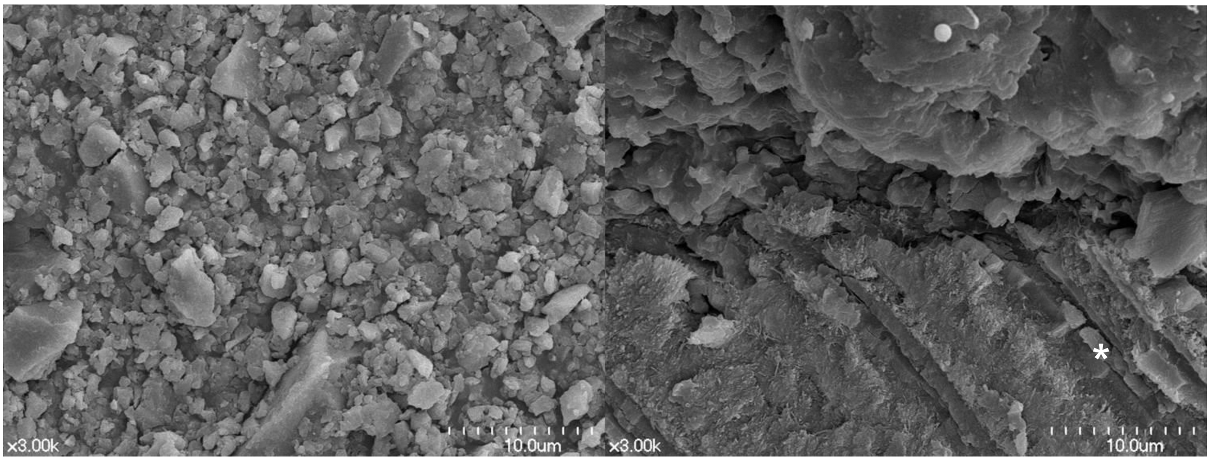
(b)


x3.00k
10.0um
x3.00k
10.0um

(c)



(d)



(e)

Figure 4. SEM images of dentinal tubules. (a) non−treated opened tubule, (b) Nd:YAG laser−treated, (c) Er:YAG laser−treated, (d) SuperSeal−treated and (e) ClinproXT−treated.

Note the melted dentin (solid arrows), oxalate crystals (broken arrows) and resin tag (star).

국문초록

레이저 조사 및 탈감작제 적용에 따른 상아세관액 흐름의 실시간 측정

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1. 목적

본 연구의 목적은 노출된 상아질에 Nd:YAG, Er:YAG 레이저 및 도포용 탈감작제를 적용하였을 때 상아세관액 흐름의 변화를 실시간으로 측정하여 상아세관 폐쇄효과를 평가하고자 하였다.

2. 재료 및 방법

우식 및 수복물이 없는 발치된 대구치 32개를 백악-법랑경계 하방 5 mm에서 절단한 후 슬라이드글라스에 접착하였다. 준비된 시편을 실시간 미세 물 흐름 측정장치(nano-Flow)와 연결 후 미세누출이 없음을 확인하였다. 치경부에 V 모양의 와동을 형성하여

상아질을 노출시킨 다음 산부식, 수세, blot-dry 후 Nd:YAG, Er:YAG 레이저 및 탈감작제인 SuperSeal, RMGI 바니쉬인 ClinproXT를 적용하고 실시간으로 상아세관액의 흐름을 측정하였다(n=8). 레이저 및 탈감작제 적용 전후의 상아세관액 흐름율을 구하여 비교하였다. 각각의 처리방법에 따른 상아세관의 상태를 주사전자현미경을 통해 관찰하였다.

3. 결과

Desensitizing 방법에 따라 서로 다른 상아세관 흐름을 보였다. 레이저와 탈감작제 적용 후 상아세관액 흐름율의 감소를 보였다($p<0.05$). 상아세관액 흐름율의 감소는 ClinproXT군이 가장 높았고(71.9%), SuperSeal(34.8%), 레이저군 순이었다($p<0.05$). Nd:YAG(24.1%)와 Er:YAG(20.6%)군은 통계적으로 유의한 차이를 보이지 않았다($p>0.05$). 주사전자현미경 영상을 통해 레이저군과 SuperSeal군에서 좁아진 상아세관을 관찰할 수 있었으며, ClinproXT군에서는 RMGI에 덮여 폐쇄된 상아세관을 볼 수 있었다.

4. 결론

본 연구에서 사용한 실시간 미세 물 흐름 장치는 레이저 조사 및 탈감작제 적용 전후 상아세관액 흐름율의 변화를 분명하게 보여주었다. 상아세관액 흐름율의 감소에 있어, RMGI 바니쉬인 ClinproXT와 oxalate 기반의 탈감작제인 SuperSeal이 Nd:YAG 및 Er:YAG 레이저보다 더 좋은 결과를 보였다.

주요어: 상아세관액 흐름, 실시간 측정, Nd:YAG 레이저, Er:YAG 레이저, 탈감작제

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